

OBSERVATIONS ON THE DIFFERENTIATION OF THE GRANULES IN THE EOSINOPHILIC LEUCOCYTES OF THE BONE-MARROW OF THE ADULT RABBIT

PRELIMINARY NOTE

A. R. RINGOEN

*From the Histological Laboratory of the Department of Animal Biology, University
of Minnesota, Minneapolis*

In a recent paper¹ the writer has described the mast myelocytes and mast leucocytes in the bone-marrow of the rabbit. No evidence could be found in support of the theory that the mast cell of the rabbit represents a young or 'unripe' eosinophil or special cell,² or for the view expressed by Pröscher that the mast granules are products of a mucoid degeneration of the spongioplasm of a lymphocyte. The preparations show that mast leucocytes are true granular cells, equivalent in all respects to the other granular cells, with both the myelocyte and fully differentiated forms represented in the marrow.

The supposed relationship of mast leucocytes to eosinophil and special leucocytes necessitated a detailed study of their development also. The same material which was used for the study of the mast leucocytes was found to be excellent for the investigation of the other granulocytes, and of these the eosinophil leucocytes were of special interest on account of the many theories regarding the origin of their granules.

The exact origin of the eosinophil leucocytes of mammals has been the subject of considerable investigation, and up to the present day there is no unanimity of opinion among investigators as to the source and nature of the granules of these cells. The literature relative to the subject is enormous; it shows that

¹ Anat. Rec., vol. 9, no. 3, 1915.

² As claimed by Pappenheim's students: Benacchio, Kardos, and Szècsi.

the most divergent theories and explanations are held with reference to the histogenesis of these cells.

Various investigators of the eosinophil problem have come to recognize that the supply of eosinophilic leucocytes in the adult animal is not necessarily limited to mitosis of pre-existing eosinophilic myelocytes, but that a heteroplastic means of regeneration of these cells must also be taken into consideration. The investigations of Tettenhamar ('93), Sacharoff ('95), Brown ('98), Weidenreich ('01), Howard and Perkins ('02), Ascoli ('04), Maximow ('09), Pappenheim ('09), Badertscher ('13), Downey ('13, '14), and Barbano ('14), have shown the importance of the heteroplastic form of development. Downey confined his studies to the differentiation of the eosinophilic leucocytes of the bone-marrow of the guinea-pig and found that heteroplastic development of these cells from non-granular cells is by no means an exceptional process.

Haematologists who believe in a heteroplastic form of differentiation of eosinophils, however, do not agree on the derivation of the granules, and consequently a number of views have been advanced which have sought to account for their source and nature. The older view, that eosinophils are formed from polymorphonuclear neutrophils by a direct transformation of their granules, has within recent years lost support. Brown ('98) has described such a direct transformation of neutrophil granules into eosinophil granules in human muscle infected with trichinae. This theory has been advanced a number of times by different investigators, but it appears that the conclusion for such a direct transformation of one type of granule into another type is not based on sufficient evidence. The mere fact that Brown found a marked increase in the number of eosinophils, with a corresponding decrease in the number of polymorphonuclear neutrophils, does not necessarily indicate that a direct transformation process was going on.

The eosinophils are so widely distributed throughout the tissues, and in certain pathological conditions become so numerous, that it seems quite reasonable to believe that they multiply in these situations by homoplastic means also, since the cor-

responding myelocytes are often present (Gulland and Goodall, Herzog).

At the present day the literature relative to the origin of the eosinophil granules during heteroplastic differentiation of eosinophilic leucocytes may be said, in the main, to be rather sharply centered about the belief that the granules are of an exogenous origin. Weidenreich is the chief exponent of this theory; he believes that the granules of all eosinophils are hemoglobin-containing products of degenerated erythrocytes,³ i.e., he believes that the granules are not the products of protoplasmic activities of the cells which contain them.⁴ Weidenreich states explicitly that this is the only source of the eosinophil granules, and furthermore, that there are no observations on record which prove a gradual differentiation of eosinophil granules in the protoplasm of non-granular cells.⁵

The theory that the eosinophil granules are of an exogenous origin, and that they are not related to hemoglobin, has been given additional support by the recent observations of Badertscher ('13), who believes that the granules of eosinophil leucocytes seen in the neighborhood of degenerating muscle fibres and erythrocytes in *Salamandra atra* during metamorphosis are products of the degenerating fibers and red cells, and that they are, therefore, related to hemoglobin or its dissociation products.

Weidenreich's hemoglobin theory has met with many staunch supporters. Downey ('13, '14), however, has recently taken exception to the theory in so far as the eosinophils of the bone-marrow are concerned. He states that his preparations showed nothing which would indicate hemoglobin was concerned in the elaboration of these granules; he believes that the eosinophil

³ Anat. Rec., 1910, vol. 4, p. 327, "Die eosinophilen Granula der Säugetiere sind als exogene Plasmaeinlagerung zu bezeichnen und zwar als hämoglobinhaltige Teile, grösstenteils von Erythrocyten herrührend, die durch hämolytische Vorgänge zerstört, oder in toto phagocytiert wurden."

⁴ Anat. Anz., 1901-1902, vol. 20, p. 197, "Die eosinophilen Leucocyten sind also nichts anderes als sog. Lymphocyten, welche die durch den Zerfall roter Blutkörperchen entstehenden feinen Trümmer in ihren Plasmaleib aufnehmen, wobei ihr Kern in die polymorphe Form übergeht."

⁵ Die Leucocyten und Verwandte Zellformen, p. 250.

granules are real intracellular formations (endogenous differentiations), which are the products of specific activities of the protoplasm. Downey's view is, therefore, in strict opposition to that of Weidenreich and others, who believe in an exogenous origin for all eosinophil granules.

Barbano ('14) has also recently favored the view that the granules are endogenous formations; he believes that they are secretory granules which may be extruded from the cells. His conclusions are based entirely on a study of local eosinophilia in various pathologic conditions.

Under normal conditions the eosinophil leucocytes have been reported by various investigators as being widely distributed throughout the tissues, appearing in great numbers in the gastrointestinal tract, in the walls of the trachea, in the connective tissue surrounding the bronchi, in lymph glands, the thymus, and hemolymph glands. Under certain conditions the number of these cells may be materially increased, so that great numbers of them may appear throughout the section. It now seems certain that many of them are the products of local development, while others have emigrated from the vessels.

The local development of eosinophils, however, is still denied by many authors. Barbano believes that, in local eosinophilia, he can exclude the emigration of myelocytes from the vessels, and that the cells which are found in these local accumulations are, therefore, new differentiations from non-granular cells. The latter are typical small and large lymphocytes. That they may differentiate into granulocytes is shown by the fact that Barbano finds many mononuclear eosinophils whose nuclei are indetical with those of the lymphocytes. That lymphocytes, especially small lymphocytes, are concerned in the production of acidophil granules was shown also by Downey and Weidenreich,⁶ Howard and Perkins,⁷ and others.

⁶ Downey, H., and Weidenreich, Fr. 1912, Über die Bildung der Lymphozyten in Lymphdrüsen und Milz. Arch. f. mikr. Anat., Bd. 80.

⁷ Howard, W. T., and Perkins, R. G. 1902, Observations on the origin and occurrence of cells with eosinophile granulations in normal and pathological tissue. The Johns Hopkins Hospital Reports, vol. 10.

The theory that the eosinophil granules are derived from phagocytosed material (Weidenreich, Badertscher, Brown, and a great many others), is based largely upon the presence of free eosin-staining granules among erythrocytes and muscle tissue which are undergoing degeneration. These free granules are believed to be ingested by lymphocytes which are then converted into eosinophils, many of which are distinctly mononuclear.⁸

Benacchio ('09), in considering the bone-marrow of the rabbit, was primarily interested in the origin of the mast leucocytes of this animal; consequently, he did not make detailed investigations as to the histogenesis of eosinophils and special cells. He concluded, however, that the myelocytes with basophilic granules which he found in great numbers in his preparations were not real mast myelocytes, but simply 'unripe' stages of young eosinophil and special cells.⁹ Pappenheim, Kardos, and Szécsi also regard all the basophilic granulocytes in the marrow of the rabbit as immature eosinophils and special cells. Many haematologists, in fact, believe that the early myelocyte stages of eosinophil and special cells have a granulation, which has a predominant basophilic element when first differentiated. These granules, however, do not retain their basophilic element, as they should if they were real mast granules, but they undergo a gradual transformation or 'ripening process,' during which they change their staining reactions and are finally transformed into eosinophil granules. That such transformation actually takes place has been reported by Ehrlich ('78, '79), Schwarze ('80), Hirschfeld ('98), Benacchio ('09), Kardos ('09), Pappenheim and Szécsi ('09), Maximow ('10, '13), and Downey ('13, '14).

The early basophilic granules of eosinophils and special cells, however, are in no way related nor similar to the basophilic

⁸ Sternberg claims (Ueber die Entstehung der eosinophilen Zellen. Beitr. z. path. Anat. und allg. Pathol., Bd. 57) that he can distinguish real eosinophil granules from erythrocyte fragments.

⁹ Mast myelocytes or fully differentiated mast leucocytes could not be found when Benacchio's methods were used. For the detection of these cells in the marrow of the rabbit, methods of technique must be used in which water is absolutely avoided. After lucidol-acetone fixation, however, the granules are more resistant to water and are able to withstand its action while being stained in watery staining combinations.

granules of mast leucocytes. The mast granules are endowed with certain specific and diagnostic characters at their first appearance within the cell body, and they are readily distinguished from the granules of eosinophils and special myelocytes. This is contrary to the statements of Weidenreich, who believes that if all of the granules of the eosinophil and special leucocytes were basophilic when first formed, it would be impossible to distinguish their myelocytes from the basophilic myelocytes of mast leucocytes. I have found, however, that mast granules can always be distinguished from the granules of other basophilic myelocytes, provided that the proper methods of fixation have been applied to the marrow.

Mention has already been made of the fact that the methods of technique employed by Benacchio failed completely to demonstrate mast leucocytes, although his preparations did show great numbers of eosinophil and special cells. These results would indicate that the chemical composition of mast granules in the rabbit, at least, is quite different from that of the ordinary basophilic granules of young eosinophils and special cells. Mast granules are more soluble in water than are the basophilic granules of either eosinophil or special myelocytes. As far as resistance to water is concerned, it also appears that the granules of the mast leucocytes of the circulating blood are quite different from the granules of the mast myelocytes and the more fully differentiated mast cells of the marrow. The granules of the mast leucocytes of the blood are less soluble in water than are the granules of the mast myelocytes and the corresponding leucocytes of the marrow. This difference in the constitution of the granules is shown by the fact that the most ordinary methods will preserve the granules of the blood mast cells, while the strict elimination of water is necessary for the preservation of the granules of both the mast myelocytes and the fully differentiated mast leucocytes found in the bone-marrow. In the older mast leucocytes, or those found in the blood stream, there may be some chemical change within the cell body—initiated by the blood plasma—as soon as the leucocytes are thrown out into the circulation, which in turn acts upon the mast granules

changing their composition to a greater or less extent and thus rendering them more resistant to the action of water. The mononuclear mast leucocytes are never found in the blood of the normal adult rabbit, but are confirmed under ordinary conditions to the marrow. In these cells the basophilic granules are very sensitive to the action of water. As the number of granules increase the nucleus gradually becomes polymorphous, while in the fully differentiated mast leucocyte of the circulating blood the nucleus is very polymorphous and the granules are comparatively resistant to the action of water.

The presence of basophilic granules in eosinophil myelocytes is no longer doubted nor questioned by haematologists, their occurrence having been reported by a number of investigators, including Arnold, Hirschfeld, Hesse, Benacchio, Kardos, and others. Maximow and Pappenheim have called particular attention to the very decided basophila of young eosinophil and special granules in the eosinophils and special cells of the rabbit.

An early 'primitive' granulation which is also basophilic has been reported by several investigators. Pappenheim regards it as an early or 'prodromal' granulation that is not related to the final eosinophil or special granulation which appears later as a new differentiation. According to Pappenheim the 'prodromal' granulation is derived from the nucleus of the cell and is basophilic; it disappears when the specific granulation appears later. He believes that the eosinophil granules are a new development and that they too are basophilic when first differentiated. Weidenreich admits the presence of basophilic granules in some of the eosinophils, but claims that they are either fragments of the nucleus or endogenous differentiations which are in no way related to the eosinophil granules. Maximow described a primitive azurophil granulation in eosinophil myelocytes, and he also claims that it is not related to the specific granulation which is developed later. Hertz and Pappenheim have also described an azurophil granulation in the leukoblasts and myelocytes of myelogenous leukemia.

As early as 1895, Arnold, in studying the morphological features of the cells of the marrow of the rabbit, observed that many granulocytes contained basophilic granules. He also noticed that many myelocytes of the same type contained both basophilic and acidophilic granules within the same cell body. Arnold thought it probable that the basophilic granules were transformed into acidophil granules, since so many of the former showed considerable variation in their staining reactions even within the same cell body. Arnold, in fact, seems to have made the correct interpretation of his preparations; however, he gave no detailed descriptions of the gradual changes in the morphology of the granules during the transformation process; neither did he work out in a detailed manner the gradual changes in the staining reactions of the early basophilic granules.

Other investigators of the bone-marrow have also noted changes in the staining reactions of the basophilic granules of eosinophilic myelocytes and have, in fact, reported a transformation of the basophilic into the acidophilic type, but they have not made a particular study of the transformation process itself and of the other phenomena which are seen to accompany it. The life-history of the eosinophil granule has not been worked out with sufficient detail to warrant the statement that all basophilic granules in eosinophilic myelocytes represent unripe eosinophil granules. No attempt has been made to give minute descriptions of the gradual changes in staining reactions which the basophilic granules pass through in becoming transformed into acidophil granules.

Maximow, in his earlier investigations of the bone-marrow, was interested primarily in the rôle which lymphocytes played in the elaboration of granules in their protoplasm, and in the regeneration of the granular leucocytes, consequently, he also failed to make a detailed study of the histogenesis of the eosinophil series. In 1913, however, his figures show that the very youngest granules of eosinophil myelocytes are basophilic when first differentiated, and that they are gradually transformed into acidophil granules, although Maximow does not emphasize this point in particular. He used cover-glass preparations (Helly

fixation followed by staining in alcoholic thionin) and found that the granules of the eosinophils showed considerable variation in their staining reactions depending on the stage of differentiation that they had attained. The older or more fully differentiated granules were stained green in the alcoholic thionin, while the myelocytes contained granules which were stained blue, in addition to other granules which were of a green color.

Downey ('13, '14) has made a special study of the life-history of the eosinophil granules based on the variations in staining reactions and form of these granules. He also finds that the eosinophil granules are basophilic when first differentiated. Gradually, however, these early basophilic granules change their staining reactions, taking on the eosin of the stain when subjected to the action of the indulin-aurantia-eosin staining combination, instead of staining in the indulin, as Downey found to be the case when the granules were first differentiated. Furthermore, he found that in the later stages of differentiation the granules lost their avidity for the eosin of the staining mixture and stained with the aurantia of the same staining combination. In regard to the staining reactions, Downey states that

Some of the granules change their staining reactions while they are still small and basophilic, while others remain basophilic until they have reached a size even greater than that of the fully differentiated granule before such change takes place. That these larger granules do not disappear, and that they are transformed directly into the eosinophil granules is shown by the fact that many of the largest ones are stained in the acid component of the staining mixture, while others are of a mixed tone.

These gradual progressive changes in the staining reactions of basophilic granules in eosinophilic myelocytes together with changes in their shape and size have led Downey to conclude that as far as the bone-marrow is concerned, eosinophil granules are true endogenous formations resulting from special activities of the protoplasm, and that they are not related to hemoglobin or its dissociation products, as maintained by Weidenreich and others.

Barbano, who also regards the eosinophil granules as real endogenous differentiations, has also noted differences in the

staining reactions of eosinophil granules, although he does not give detailed descriptions of these changes. In using the hemalum and eosin staining combination, he found that there were great individual differences in the avidity with which the granules stained in the eosin. In an epithelioma of the uterus, also in other similar cases, Barbano found that many of the granules in cells of the lymphocyte type stained only slightly in the eosin, while others scarcely stained at all with this dye. The latter appeared clear and refractive and were colored by only the slightest tinge of eosin. Barbano, however, does not interpret these differences in staining reaction as indicating a gradual 'ripening' process of the eosinophil granules. He believes that lymphocytes under certain conditions differentiate granules which are typical eosinophil granules from the very beginning, although in some instances the early formed granules may not exhibit a remarkable affinity for the acid component of the staining mixture.

Downey, however, has shown that the first granules of the eosinophil myelocytes are not the typical granules of the fully differentiated cells. He believes that the fully differentiated granule is the end product of a series of gradual, complex changes in chemical constitution, as well as in form and size, of the small 'unripe' granule which first appeared in the protoplasm of the myelocyte.

In view of the varied opinions concerning the origin and nature of the eosinophil granules, and since the majority of those authors who believe in the hemoglobin nature of the granules have based their studies on local eosinophilia, it is of importance that new studies of the bone-marrow be undertaken, with their results in mind, in order to determine whether the eosinophils of the marrow develop under conditions which might indicate that their granules are also related to hemoglobin products. Fortunately I have had the great pleasure of carrying on an investigation of this kind under the direction of Professor Downey, to whom I am greatly indebted. As far as the marrow of the rabbit is concerned, my observations on the life-history of eosinophil granules do not differ essentially from those of Professor

Downey. In strict corroboration with his findings, my preparations showed nothing which would indicate that hemoglobin was a contributing factor in the formation of these granules.

Many of the myelocytes, in smears prepared according to Pappenheim's method,¹⁰ contain basophilic granules only. These cells might easily be taken for mast myelocytes if it were not for the fact that other similar cells contain oxyphilic granules also. The oxyphilic granules may be very numerous or there may be only a few of them in any one cell, and, in general, the presence of a greater number of oxyphilic granules in a cell seems to be conditioned on a corresponding diminution in the number of basophilic granules. This, together with the fact that many of the granules are intermediate in staining reaction, shows that there is a gradual change in the staining reaction of the granules from basophilic to oxyphilic. The intermediate stages in this gradual process of 'ripening' are so numerous that there is no question but what all of the basophilic granules seen in preparations prepared according to this method are eventually transformed into granules whose chemical constitution becomes such that they finally stain only in the acid component of the staining combination. Such granules are surely not mast granules, for the latter have never been known to change their staining reactions in this way. They remain basophilic throughout their existence.

No other type of basophilic granules, besides those which eventually become oxyphilic, could be found in the preparations prepared by the above mentioned method. We must, therefore, give Benacchio credit for a correct interpretation of the nature of these granules when he stated that the cells which contain them are young 'unripe' eosinophil and special leucocytes. Benacchio, however, did not go into the details of the gradual differentiation and transformation of these granules, and such detailed study would have been impossible with his methods, because with them the vast majority of the cells are distorted, their granules are swollen, and the outlines of the nucleus are usually indistinct.

¹⁰ *Folia Haem., Archiv, Bd. 13.*

The results obtained by Kardos ('09), in working with sections of bone-marrow of the rabbit fixed in 100 per cent alcohol and in Helly's mixture, are difficult to understand. He found neither mast cells, nor cells of any kind which contained basophilic granules. Contrary to the findings of Kardos, I find that in sections of bone-marrow (material fixed in 100 per cent alcohol and stained in alcoholic thionin) myelocytes with basophilic granules are very numerous, and furthermore, that in these same preparations it is also possible to demonstrate mast myelocytes and fully differentiated mast leucocytes. Sections stained in May-Giemsa not only show many granulocytes which contain basophilic granules, but also other cells in which both basophilic and acidophilic granules are intermixed, and still others in which all of the granules are of the acidophil type. The preparations also show the various other types of granulocytes which are characteristic of the marrow of the rabbit. The basophilic granules of the eosinophil and special myelocytes are also seen in sections of material fixed in Helly's fluid. True mast granules, however, are not preserved by this method, and with their granules dissolved it is difficult to identify the mast cells.

From the above it is seen that it is not a difficult matter to demonstrate basophilic granules in the marrow of the rabbit even with the most ordinary methods. These granules, however, are not the mast granules. If the latter are also desired it is necessary to avoid the use of fixing fluids which contain water. For material fixed in bulk absolute alcohol proved most satisfactory, and for smears the lucidol-acetone method of Szécsi.

Of the various methods tried for working out the life-history of the eosinophil granule, none gave sharper and more decisive results than did Benacchio's method of staining bone-marrow smears in a mixture of indulin-aurantia-eosin. These smears were fixed in Helly's fixative for fifteen minutes and then washed in running water from three to four hours. The preparations were dehydrated and finally stained in the indulin-aurantia-eosin mixture, in a thermostat at a temperature of 38°C. This gave excellent results for the study of both the eosinophil and special myelocytes. The chief advantage of this method is that it

practically eliminates the special myelocytes from our consideration, at least in the later myelocyte stages, since the granules of the special cells at no time in their evolution show any great affinity for the eosin of the staining mixture. The vast majority of the special cells have dark-grayish-black granules, but in the youngest myelocytes these granules also have a slight affinity for the eosin of the mixture, a condition which often makes it difficult to distinguish the earliest eosinophil myelocytes from those of the special cells. The special granules, however, very soon develop their strong affinity for the indulin to the complete exclusion of the eosin, while many of the granules of the eosinophil myelocytes become strongly oxyphilic, causing them to stain intensely with the eosin.

In the earlier stages of the eosinophil myelocytes in which there are only a few acidophil granules, there are a great many small basophilic granules, with a few medium-sized and large basophilic granules scattered among them. In the later myelocyte stages, however, most of the granules are large and many of them are acidophilic. However, the later stages, including those in which most of the granules are acidophilic, contain a few small basophilic granules as well as a few larger ones. It is very probable that these smaller granules are the youngest ones; all of them probably increase in size before developing an affinity for the eosin of the stain. The presence of the small indulinophilic granules in the later myelocyte stages in which the eosinophilic granules are very numerous could not be accounted for by Hirschfeld. Downey, however, believes that these smaller granules represent recent differentiations, since they are small and still basophilic.

In addition to the changes in the staining reactions of young eosinophil granules which at first are basophilic, there is further evidence in favor of the view that these young basophilic granules are the precursors of eosinophil granules. The life-history of the eosinophil granule, in the rabbit at least, is not completed with the change in staining reaction, since the granules in the fully differentiated eosinophil leucocytes of the blood of this animal are typically spindle shaped, while the early granules

are spherical. Downey has already shown (as far as the eosinophil leucocytes of the guinea-pig are concerned, and the histogenesis of these cells seems to be very similar in the bone-marrow of the rabbit) that in addition to the changes in the staining reactions there are further changes in the morphological features of these granules which prove conclusively that the basophilic granules are in reality the younger eosinophil granules.

The granules of both the eosinophil and special leucocytes are very small when they are first formed, and both types of granules are stained dark with the indulin, with possibly a slight tinting with the eosin of the mixture. This uniformity in size and staining reaction frequently make it impossible to distinguish the earliest special myelocytes from those of the eosinophil leucocytes. Maximow encountered the same difficulty in rabbit embryos, but claims that he could always distinguish the two types of cells in the marrow of post-natal animals. In the latter he finds that the first eosinophil granules are from the very beginning brighter and coarser than are those of the special cells; at first the youngest granules possess a clear basophilic quota and stain a bluish tinge, but are not metachromatic as are the granules in the special cells. In spite of these slight differences there are times when it is almost impossible to classify basophilic myelocytes with any degree of certainty. Maximow admits that in alcoholic thionin preparations it is very difficult to distinguish between eosinophil and special myelocytes. He states, however, that the granules of the eosinophils can often be distinguished from the granules of the special cells by the fact that some of the eosinophil granules enlarge very rapidly "und dass man infolgedessen schon in den noch ganz granulaarmen Zellen typische, grobe, glänzende azidophile Granula neben nur sehr spärlichen feineren erblicken kann."

Downey also states that when the granules are few in number and of small dimensions there may be considerable difficulty in distinguishing between eosinophil and special myelocytes. He also found that the granules of eosinophil myelocytes enlarged shortly after they were differentiated and that some of them changed their staining reactions, while others remained basophilic

for a longer period of time. The basophilic granules in young special myelocytes, on the other hand, remained basophilic for a longer period of time which was followed by a rapid change in staining reaction involving all of the granules.

The same difficulty in determining the exact position of the very earliest myelocytes, i.e., those with a few granules only, was encountered in the present investigation.

In the indulin-aurantia-eosin preparations there is no difficulty in finding basophilic myelocytes which contain a few decidedly oxyphilic granules. Myelocytes containing the latter can be diagnosed as eosinophil myelocytes, since the granules of the special cells are never stained intensely with the eosin of the mixture. It must be admitted, however, that there were always a few cells which it was difficult to classify. With more intensive study it might be possible to properly place these cells also. However, it is not the object of the writer to describe the morphological features of the very earliest myelocyte stages of eosinophil and special myelocytes. The indulin-aurantia-eosin preparations show beyond all doubt that, in so far as the adult animal is concerned, the bone-marrow contains two distinct types of myelocytes, the precursors of eosinophils and special cells respectively. The inability to diagnose the specific type of basophilic myelocyte in every case—before some of the granules stain in the eosin—does not invalidate the conclusions in regard to the life-history of the eosinophil granules.

The results of the study outlined above show that these granules are differentiated gradually from the basophilic protoplasm of non-granular cells, and that they pass through a gradual progressive development which is expressed in changes in staining reaction, as well as in shape and size. When first formed they are indulinophilic (with Ehrlich's triglycerine mixture) or basophilic with heterogeneous mixtures containing a basic dye. At first there are only a few granules in the cell, but their number is gradually increased, the youngest granules always being basophilic (or indulinophilic), while the older ones have become distinctly acidophilic. The number of granules which are intermediate in staining reaction, i.e., which have an affinity for

both the acid and the basic component of the heterogeneous mixtures (or for indulin and eosin of the triglycerine mixture), shows that there is a gradual transformation from one type of granule to the other, and not a replacement of one kind by another. There is no evidence for Weidenreich's view that the basophilic granules, which he admits are present in the myelocytes of eosinophil leucocytes, are endogenous formations which are not related to the eosinophil granules which are supposed to be developed later from products of hemoglobin dissociation.

The changes in the character of eosinophil granules are similar to those which are seen during the development of the special granules. For the latter these changes are universally conceded to indicate a process of gradual differentiation, progressive development and 'ripening.' It is difficult to see why the same conclusion should not apply to the eosinophil granules, especially when there is nothing to indicate that in the normal bone-marrow fragmenting erythrocytes or other hemoglobin products are in any way concerned in their development.

The development of eosinophil leucocytes in the tissues is a different question. For them there is much evidence to show that their granules are closely related to hemoglobin products. Their granules apparently do not pass through the same series of changes during their development as was outlined above for the eosinophil granules of the bone-marrow, at least no such changes have ever been described. This may be due to the fact that local eosinophilia has never been investigated from this same standpoint. Barbano's recent study of the subject indicates merely that the granules of the tissue eosinophils are quite variable in their staining capacity with eosin. These variations, however, do not seem to be bound with any particular stage in the development of these leucocytes, and there was nothing to show that those granules which had the least affinity for the eosin were the youngest ones. Barbano, however, has peculiar ideas about eosinophil leucocytes and his results do not seem very trustworthy.

Gütig believes that there are two types of eosinophils, those of the tissues and those of the marrow and blood. The granules of the hematogenous eosinophils are true endogenous differenti-

ations, while those of the tissues are of an exogenous origin. Downey agrees with this conclusion, provided that the observations of Weidenreich and others on the development of eosinophil granules in local eosinophilia are correct. With these questions in mind a renewed study of local eosinophilia would be of great importance.

SUMMARY

The bone-marrow of the normal adult rabbit shows no evidence for the support of the theory that the eosinophil granules are exogenous formations which are derived from hemoglobin or its dissociation products (Weidenreich and others).

Application of the proper methods of technique, however, show that these granules are real manifestations of protoplasmic activities, and that they are gradually differentiated in the cytoplasm of mononuclear cells (Downey).

The indulin-aurantia-eosin preparations show that the youngest granules of the myelocytes are indulinophilic. They do not remain indulinophilic for any length of time, but pass through a series of progressive evolutionary processes during which they change their shape and staining reactions. Finally they are transformed into typical eosinophilic granules. In the fully differentiated eosinophil leucocyte of the marrow all the basophilic (with Giemsa, etc.) granules have been converted into acidophilic granules and no new basophilic (indulinophilic with triglycerin) granules are formed.

These gradual changes in the staining reactions and morphology of basophilic granules in the myelocytes of eosinophil leucocytes must be interpreted as indicative of progressive evolution on the part of the eosinophil granules.

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MEMOIRS
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No. 6

THE RAT

COMPILED AND EDITED BY
HENRY H. DONALDSON

REFERENCE TABLES AND DATA FOR THE ALBINO RAT (*MUS*
NORVEGICUS ALBINUS) AND THE NORWAY RAT
(*MUS NORVEGICUS*)

To be published in October

Cloth bound. Price, post paid to any country, \$3.00

PREFACE

For a number of studies on the growth of the mammalian nervous system made by my colleagues and myself we have used the albino rat. In the course of the work we frequently felt the need of referring to other physical characters of the rat to which the nervous system might be related. This led us to collect such data as were already in the literature and also led us to make further investigations. The facts gathered in this way have proved useful to us and are here presented in the hopes that they will be useful to others also.

CONTENTS

Preface. Introduction. Classification. Early records and migrations of the common rats.

Part 1. Albino rat—*Mus norvegicus albinus*. Chapter 1—Biology. Chapter 2—Heredity. Chapter 3—Anatomy. Chapter 4—Physiology. Chapter 5—Growth in total body weight according to age. Chapter 6—Growth of parts or systems of the body in weight. Chapter 7—Growth of parts and organs in relation to body length and weight according to age. Chapter 8—Growth in terms of water and solids. Chapter 9—Growth of chemical constituents. Chapter 10—Pathology.

Part 2. The Norway Rat—*Mus norvegicus*. Chapter 11—Life history. Chapter 12—Growth in weight of parts and systems of the body. Chapter 13—Length of tail and weights of body, brain and spinal cord in relation to body length. Chapter 14—Growth in terms of water and solids. Chapter 15—References to the literature. Index.

AN ABNORMAL FROG'S HEART WITH PERSISTING DORSAL MESOCARDIUM

JAMES CRAWFORD WATT

Department of Anatomy, University of Toronto

SIX FIGURES

The frog forming the subject of description in this paper belongs to the species *Rana pipiens*, bred in tanks in the Medical Building of the University of Toronto. After pithing, the sternum was cut away to expose the heart, by a student working in the Pharmacological Laboratory and the abnormal heart was so striking in appearance that the frog was sent to Prof. J. Playfair McMurrich who kindly turned it over to me for investigation.

After observing the beating of the heart, the frog was placed in strong formalin solution, and the heart was injected with warm wax. The wax on hardening enabled the necessary dissection to be easily performed, and was easily removed from the heart later, to admit of examination of the cavities. The heart and great vessels were also dissected in a couple of normal frogs for purposes of comparison with this abnormal specimen.

After opening the pericardium the heart is seen (fig. 1) as a long tubular organ extending forward under the floor of the mouth, with its anterior portion displaced to the left of the median line by the hyoid bone and mass of the tongue.

The sinus venosus is situated in its usual location and receives the postcaval and the two precaval veins. It extends forward to open into the right atrium which lies directly cephalad of it. The atria are only incompletely divided from each other on the surface, and they are marked out from the sinus venosus at one end, and the ventricle at the other, by constrictions in the wall. They are of large capacity and appear as long as the whole heart

of a normal frog of equal body size, and form one-half the total length of this heart. They have an oblique position, being nearer the median line posteriorly, as they are forced out to the left side under the floor of the mouth as they proceed forward. The groove on the ventral surface, marking off the left atrium from the right, starts just cephalad of the sinus venosus on the left side, and runs obliquely forward toward the origin of the conus arteriosus, but is lost before reaching this point, so that there is no visible division externally of the atria in the part nearest the ventricle.

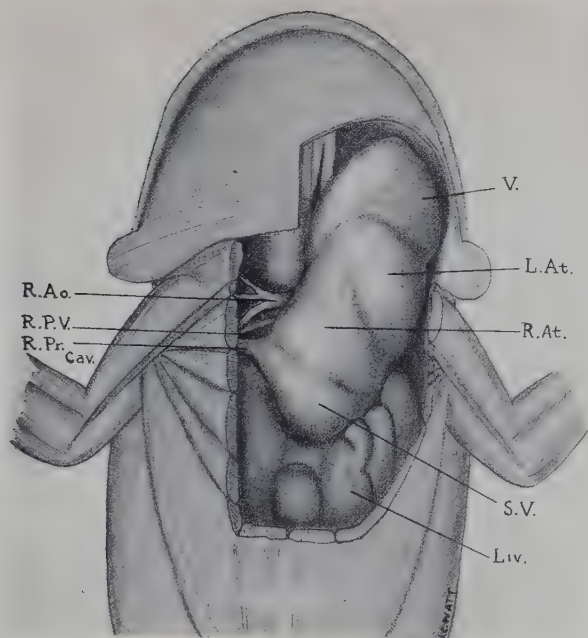
The ventricle continues forward in the line of the atria and is of normal size. Its apex is situated almost in contact with the inner side of the mandible. No conus arteriosus and no aorta can be seen with the heart undisturbed, but by lifting the atria and ventricle slightly, and pulling them out to the left, the conus arteriosus is seen leaving the ventricle on the right side, and running medially and dorsally (fig. 2). Immediately back of the hyoid bone it reaches the left side of the esophagus and bifurcates, sending the left ventral aorta directly dorsally alongside the esophagus, where it divides, and the right one horizontally across the esophagus, after which it divides, giving off the

ABBREVIATIONS

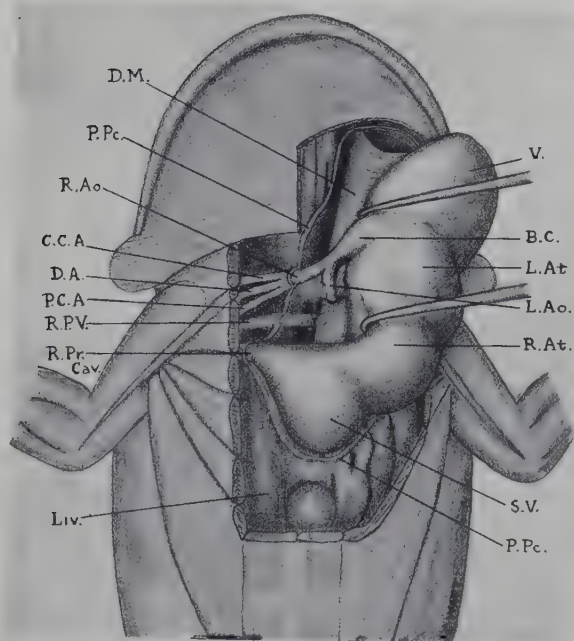
<i>Ao.</i> , aortae	<i>P.C.A.</i> , pulmocutaneous artery
<i>At.</i> , atrium	<i>Post.Cav.</i> , postcaval vein
<i>B.C.</i> , bulbus cordis or conus arteriosus	<i>P.Pc.</i> , parietal pericardium
<i>C.C.A.</i> , common carotid artery	<i>R.</i> , right half of pericardial cavity
<i>D.A.</i> , dorsal aorta	<i>R.Ao.</i> , right aorta
<i>D.M.</i> , dorsal mesocardium	<i>R.At.</i> , right atrium
<i>L.</i> , left half of pericardial cavity	<i>R.Pr.Cav.</i> , right precaval vein
<i>L.Ao.</i> , left aorta	<i>R.P.V.</i> , right pulmonary vein
<i>L.At.</i> , left atrium	<i>S.V.</i> , sinus venosus
<i>Liv.</i> , liver	<i>V.</i> , ventricle
<i>L.Pr.Cav.</i> , left precaval vein	

Fig. 1 Dissection of frog to show abnormal heart. Sternum and ventral muscles have been cut away, also the parietal pericardium; the heart lies undisturbed.

Fig. 2 Same as figure 1 except that heart has been drawn over to left by hooks, to show persistent dorsal mesocardium attached to its dorsal surface. Cut edge of parietal pericardium is also shown.



1



2

common carotid trunk, the rest turning dorsally to form the pulmo-cutaneous artery and dorsal aorta. No abnormalities exist beyond this point, all the arterial trunks appearing normal.

When the heart is lifted a structure which is probably the mechanical cause of the abnormality comes into view. This is a double fold of pericardium (fig. 2) reflected from the wall of the sac to the dorsal surface of the heart. It extends all the way from the sinus venosus to the apex of the ventricle, and at this latter point exhibits a free edge. Running off at right angles to this fold as it lies over the ventricle, is a process of the fold passing to the right over the conus arteriosus and ventral aortae, right out to where these latter structures leave the pericardial cavity, so that this smaller fold has no free border.

I interpret this membrane as a completely persistent dorsal mesocardium. Its presence may be the cause of the abnormal shape of the heart, for the membrane normally disappears as flexion begins in the heart tube, and its continued existence would I think, be an obstruction to this flexion, especially to the bend which would project the heart toward the ventral surface (see arrow, fig. 3). It would thus hold the heart in the extended tubular condition. There has been a certain amount of flexion, however, in this heart, shown by the conus arteriosus coming off the ventricle dorsally and running caudally and medially. The free anterior border of the dorsal mesocardium is thus accounted for, as that part of the membrane lying in the long axis of the heart represents the mesocardium only up to the point of flexion which forms the apex of the ventricle. The mesocardium (fig. 6) originally anterior to this point has been carried back with the conus arteriosus, over which it is still found, and now appears as a process running off from the right side of the rest of the membrane.

The presence of this flexion seems to be an argument against regarding the mesocardium as a force resisting the folding up of the heart tube, but on analysis of the nature of the bend exhibited, this objection is found to be more apparent than real. The bend which has occurred, it will be remembered, is one which brings

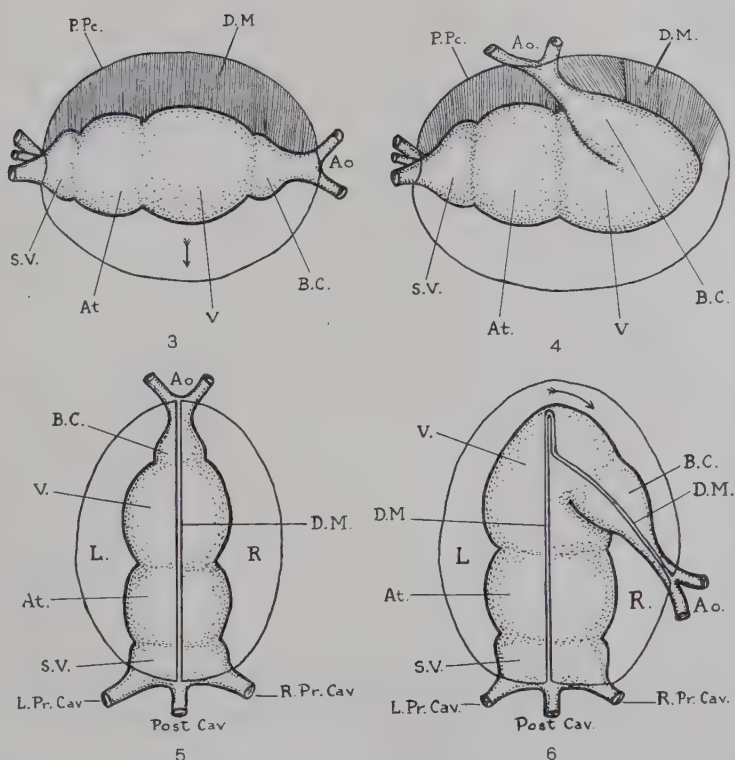


Fig. 3 Diagram of normal unflexed heart tube seen from the right, lying in pericardial cavity, showing dorsal mesocardium. Movement of heart tube in direction of arrow would be resisted. (See under figures 1 and 2 for list of abbreviations).

Fig. 4 Diagram of abnormal frog's heart seen from right, lying in pericardial cavity and showing persistent dorsal mesocardium.

Fig. 5 Diagram of normal unflexed heart seen from above to show line of attachment of dorsal mesocardium.

Fig. 6 Diagram of abnormal frog's heart seen from above, to show line of attachment of persistent dorsal mesocardium. Arrow indicates direction in which flexion has occurred.

the part of the tube displaced, slightly above and to one side (figs. 4 and 6) of the part still retaining its original position. This means that no stretching of the suspending membrane of the tube—the mesocardium—is necessary, but only a folding of this layer on itself to one side. There is no increase in the distance between any part of the heart and the roof of the pericardium. There would of necessity be a great increase in this distance over a part of the heart if the fold had occurred which projects the ventricle (see arrow, fig. 3) into a position ventral to the atria, and this would of course bring great strain and stress to bear on the mesocardium, in order to stretch the part attached to the regions of the heart involved, sufficiently to permit of the flexion. As the mesocardium forms a continuous double-layered membrane suspending the whole heart tube, its strength would be much greater than any adventitious bands or adhesions. Its thickness, also is considerable, relatively to the size of the heart in early stages of development, and so I think it is probable that it was possessed of sufficient strength to resist stretching, and so to prevent the normal atrioventricular bend. The fact that the conus bend occurred was because it could take place without stretching the attached membrane, merely folding it on its side.

The left pulmonary vein comes directly into the left atrium through the dorsal mesocardium. The right vein runs across the roof of the pericardial cavity (figs. 1 and 2) in which it forms a transverse fold lying caudal and parallel to the right ventral aorta, and in this fold it runs to the mesocardium which it enters to reach the atrium.

The flexion of the anterior portion of the heart can be accounted for by the recession of the branchial region during development, carrying back the aortic arches, and necessitating a bending back of the arterial end of the forwardly directed tubular heart. This bend is in the normal direction for that always found between the ventricle and conus arteriosus, but appears on the dorsal surface instead of the ventral because of the absence of the atrioventricular bend, which would have projected all the heart cephalad of it to the ventral surface.

From the foregoing description it will be seen that there is nothing in the condition of this heart that cannot be explained on embryological grounds. The primary cause of the deformity appears to be the failure of the dorsal mesocardium to disappear after the formation of the simple tubular heart. Development in the heart tube has proceeded in a normal way throughout, and the final form of the heart has been influenced almost solely by the mechanical action of the dorsal mesocardium, accompanied by the recession of the branchial arches.

Of course there is another explanation for the condition found here. It is that there was a primary failure of the atrioventricular region to undergo any flexion, and that the presence of the dorsal mesocardium is a second and distinct anomaly, not related in any way to the failure of flexion to occur. Both of these abnormalities are exceedingly rare. As far as I have been able to ascertain neither of these conditions has been previously described, and from the previous discussion I do not see why they cannot be regarded as cause and effect.

I have tried to find accounts of any similar conditions recorded in the literature on the heart and pericardium. Anomalies of the pericardium mentioned are fringes, apertures communicating with the pleural cavity, also complete absence of the pericardium. I have seen no description of a persistent mesocardium or any membranous septum or band which could be interpreted as such. Hundreds of cases of cardiac abnormalities are listed, but none of them were due to incomplete flexion of the heart tube. I have not read all the papers entitled simply 'Cardiac anomaly' as there are scores of them, and experience showed that what were investigated were common occurrences, such as failures of septa, and the like; thus I may possibly have missed a case similar to the present.

There is no doubt, however, that both the conditions here present are extremely rare. Both the disappearance of the mesocardium and the flexures of the heart tube are quite early occurrences in embryonic life. Usually, of course, the earlier a process occurs, the more fundamental it is, the more likely it is not to present abnormalities, being more firmly impressed on

the organism, and the graver are the consequences of departures from the normal. If the supposition is correct that the dorsal mesocardium would offer mechanical opposition to flexion in the heart, then its persistence is fraught with much graver consequences than at first thought would be supposed, and will lead to most extreme cardiac deformity. The deformity in the frog was not incompatible with full activity and functional efficiency, but it is conceivable that in higher animals it might be incompatible with existence, at least beyond fetal life. An homologous deformity in man would postulate a heart tube partly at least located in the neck, subject to compression and to stretching from the movements of this part of the body, and lacking all protection from the outside, such as is afforded by the ribs.

If the dorsal mesocardium should persist, and yet permit of flexion going on normally in the heart, it should be found in the adult as a membrane forming a complete cephalocaudal partition across the sinus transversus of the pericardial cavity, and I have seen no mention whatever of any membrane in this region. Text-books of embryology and of pathology pay scant attention to the mesocardium, unite in describing its very early and complete disappearance, and recount no case of its persistence in whole or in part. It is evidently worthy of consideration in view of the fact that it can be retained and may have far-reaching effects on the heart.

In conclusion I wish very heartily to thank Professor Henderson and Professor McMurrich, through whose kindness this interesting specimen has come into my possession: and for friendly criticism modifying the opinions expressed in this paper I am also indebted to Professor McMurrich.

April 21, 1915

A MECHANICAL DEVICE TO SIMPLIFY DRAWING WITH THE MICROSCOPE

RAPHAEL ISAACS

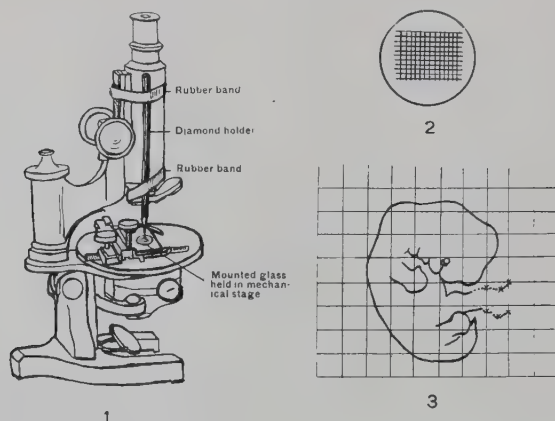
From the Anatomical Laboratory, University of Cincinnati

THREE FIGURES

It is often desirable in making rapid drawings of objects under the microscope, to have some inexpensive mechanical aid, especially when the outline is complex. For some purposes the camera lucida is either inconvenient or undesirable, being too expensive for use in large classes, besides giving a limited field. A simple accessory to aid in making fairly accurate outlines of any desired magnification, from note-book to chart size regardless of the size of the field, should thus find a place in the laboratory equipment, especially if at the same time it can be furnished at a low cost.

The device here described makes use of the fact that the mapping out of the outlines of an object is easier if some fixed points of reference on the specimen can be established and related to corresponding points on the drawing paper. These requirements will be fulfilled if a piece of glass, ruled in squares (fig. 2) is placed in the eyepiece of the microscope so that the squares appear superimposed on the object. The enlarged outlines of the object can then be made on a similarly ruled sheet of drawing paper, by noting the position at which any point of the object appears in the squares in the microscope and placing a mark on the corresponding square and position on the paper. The squares on the paper are lightly ruled in pencil and therefore easily erased. The magnification of the drawings can be regulated at will by making squares of appropriate size on the paper. To rule the squares accurately on a small piece of glass, a mounted 'writing diamond' (a small diamond mounted at the end of a piece of wood or metal) is necessary, although no doubt a carborundum pencil or other substitute may be used. The diamond, in its holder, is fastened at the side of the body tube of a microscope with two wide rubber bands (fig. 1) so that although firmly held, the diamond will have some freedom when pressure is used. The microscope is used merely as a convenient way of holding the diamond so that it can be raised or lowered without changing its position, or throwing it out of adjustment. The lines are ruled by moving the glass beneath the point of the diamond with a mechanical stage. This makes it possible to secure lines at right angles to each other and squares correct to the tenth of a millimeter. The following procedure will be found advisable:

A smooth, preferably thick piece of glass is selected and fastened to a slide with a drop of thick xylol-balsam which has been evaporated and melted on the slide. On pressing the glass down so as to insure a flat upper surface, the balsam soon cools and sets firmly. If the upper surface slants or is uneven, the ruled lines will not be of uniform thickness. A thick round cover-glass or a square cut from a slide can be used for this purpose. The slide, carrying the glass to be ruled is now fitted tightly into the mechanical stage so that there is very little free movement. By using the vernier to mark off the distances, lines



Figs. 1-3 Method of using the mechanical stage for ruling an eyepiece scale to be used in making drawings with the microscope.

Fig. 1 Microscope, with diamond holder in position.

Fig. 2 Ruled glass.

Fig. 3 Method of mapping out in drawing.

can be ruled one millimeter apart (a convenient distance for most purposes) accurate to the tenth of a millimeter. To rule the lines, the diamond is lowered with the coarse adjustment and brought into contact with the slide using very light pressure with the fine adjustment (fig. 1). The reading of the fine adjustment, when the conditions are ideal, will help to give the same pressure for every line. The pressure is tested by starting the line outside the field to be ruled. The elasticity of the rubber bands will give the diamond enough freedom to overcome any slight unevenness of the glass. The slide is moved using the mechanical stage with a steady, sweeping motion. The diamond is then lifted with the fine adjustment, and the slide brought back to the starting position, the slide being moved up and the new position adjusted until the reading on the vernier shows it to be correct. The horizontal lines should be made by moving the slide towards the side

having the fixed support, thus giving it the backing of the solid shoulder of the mechanical stage. For the same reason, vertical scratches should be made by pushing the slide forward. To make the lines at right angles to the ones first drawn, the pressure of the diamond must be as light as possible, as too great pressure in crossing scratches is not considered good for the diamond. It is unwise to go over a line once drawn, as this will often start cracking of the surface. Care should be used in removing the glass, as it is liable to break. The balsam is warmed and when soft the glass is slid off. It should then be mounted with the ruled surface exposed, on another piece of glass of a size to fit neatly into the eyepiece, using melted balsam. The ruled lines may be made more distinct by rubbing them lightly with a lead pencil, so that some of the black graphite is deposited in the grooves. In case it is desirable to cut the ruled glass into smaller pieces, special lines slightly deeper should be ruled along the places to be broken, as it is unsafe to try to break the glass along the light lines already ruled.

The whole operation of ruling takes but a short time, and a good slide can be finished in less than five minutes. With a little practice, especially if the diamond is good, the lines will be fairly even. In a properly adjusted eyepiece, the lines will be in focus, when the ruled glass is placed on the diaphragm inside, the ruled surface being placed facing downward. The glass can be used equally well in a compound microscope or in a binocular. In the latter, where a single picture of a solid object is desired, it is well to put the ruled glass on the side of the eye usually used with the ordinary microscope. Of course the two tubes will give different pictures.

To use the glass in drawing, squares of any desired size are lightly ruled on the drawing paper. It is usually simpler if the squares on the paper appear the same size as those in the microscope. Students can get good results if they use the corner of a slide as a right angle and measure the distance on the lines with a ruler. The approximate position of the object is noted on the squares in the microscope and the corresponding position found and mapped off on the squares on the paper (fig. 3). For low-power work, the lines appear better if the light is cut down. Further details of the drawing can be filled in later and the squares erased. As these squares are only a help in drawing, and as the process may tend to become mechanical, the instructor should be somewhat reserved in placing it freely in the hands of elementary students, where the efforts to draw a microscopic object are often an aid in analysing the specimen. Under high power, however, the squares are a decided help in analysing a complex field and the real educational value of the specimen comes in the synthesis of the parts in the finished drawing.

MEMOIRS
OF
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
No. 5

THE DEVELOPMENT OF THE ALBINO RAT, *MUS NORVEGICUS ALBINUS*

G. CARL HUBER

From the Department of Anatomy, University of Michigan, and the Department of Embryology, the Wistar Institute of Anatomy and Biology

I. FROM THE PRONUCLEAR STAGE TO THE STAGE OF MESODERM ANLAGE; END OF THE FIRST TO THE END OF THE NINTH DAY

THIRTY-TWO FIGURES

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II. ABNORMAL OVA; END OF THE FIRST TO THE END OF THE NINTH DAY

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A SIMPLE FORM OF DRAWING APPARATUS¹

ALEXANDER S. BEGG

From the Harvard Medical School, Boston

ONE FIGURE

This apparatus was designed for use by students in the Harvard Embryological Laboratory, where it has served with success during the courses just closed. It has effected a saving in time spent on outlines, and a gain in accuracy. By the addition of an inexpensive lens to our ordinary set of microscope objectives, it has also been utilized in neurology. The apparatus has the advantages of simplicity, cheapness, and freedom from the care and dirt of carbon arc lamps. No special room or electrical connections are needed, since it is attached to an ordinary socket in the open workroom, the back of the box being towards the windows. It must be understood, however, that it is not designed to be used in place of an Edinger, or similar apparatus for higher powers.

The apparatus (fig. 1) consists of a box, blackened on the inside, measuring 32 inches in height by 18 inches in width and depth. One side is left open, and in the center of the upper end is a hole for the reception of the main condenser system. This system consists of two plano-convex lenses, 3 inches in diameter, mounted in a cell around the upper rim of which is a flange. The cell hangs by the flange in the aperture mentioned above. The focal length of the combination is 2 inches. Above the condenser is held a Mazda projection bulb of 100 watts, mounted in an Edison keyless socket at the end of a horizontal tube, through which the conducting cord passes. This tube is held by means of a right angled sliding body on a perpendicular rod, which is mounted on top of the box; this gives an adjustment of the light source in all directions. It is found better to turn light off and on at wall fixture, and to avoid disturbing lamp when once adjusted in optical axis. A cheap tin shield around the lamp prevents light from escaping into the room.

Inside of the box are two cleats, one on either side, to support a wooden rack or frame upon which rests the stage and microscope. These cleats (b) are so placed that the upper surface of the microscope stage is 5 inches from the lower surface of the condenser. The stage is 6 inches square, with a center opening of $2\frac{1}{4}$ inches, which may be

¹ This apparatus was designed during the fall of 1914 while working under a grant from the Carnegie Institution of Washington, D. C.

reduced by a washer to $\frac{3}{4}$ inch. Beneath the stage is fixed, in inverted position, the arm and body tube of a microscope. The one pictured is from a discarded instrument of the type found lying idle in most laboratories. It has a coarse adjustment by means of the slip tube and sleeve, and a fine adjustment by micrometer screw. When once the optical axis is found, the rack is prevented from being displaced backward by means of small screws inserted behind the ends of the rack and into the upper surfaces of the cleats. This merely acts as a check to

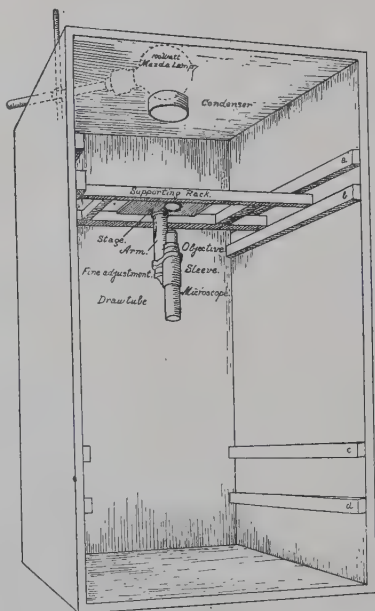


Fig. 1

backward movement, and permits removal of rack from the box in front. When working with large slides, as of brain stem, the larger stage opening is used, and in order to cover the slide, the rack and stage are moved to the upper cleats (a) about 2 inches closer to the condenser.

The lenses used are microscope objectives of 48, 32, 25, 4, 16 and 6 mm. focus, without eyepieces, and a special achromatic lens of $4\frac{1}{2}$ -inch focus, obtained locally from Pinkham and Smith, for neurological work. The mounting of this lens is threaded to fit the eyepiece end of the body tube. A 3- or 4-inch diaphragm of cardboard may be held in place by objectives to cut out rays from around object and tube. No

curtains have been used, since the body of the worker blocks the light from without fairly well.

An accessory condenser is mounted above the stage. This is a single lens of 2-inch focus which is adjustable up and down a pillar by means of a sleeve. This, however, is not needed in the ordinary routine and may be omitted. The image is received upon paper placed either upon the lower end of the box, or upon a blackened compo-board shelf placed on either of the lower cleats, depending upon the magnification desired.

The cost of each apparatus, based upon figures for the lot of eight, in use in this laboratory, is as follows:

Boxes, painting, racks, etc.	\$3.00
Main condensers, in flanged cell.	2.50
Accessory condenser in ring.	1.00
Brass stage and washer, with pillars for accessory condenser, etc. .	4.50
100-watt Mazda projection bulb.	1.50
Lamphouse, tubing, socket, cord and plug.	2.85
Special 4½-inch lens in threaded mount.	2.50
Total.	\$17.85

After the above article was in the hands of the printer, we had an opportunity to try the 250-Watt and 400-Watt gas-filled bulbs as a source of light. These lamps are highly satisfactory, the higher power light being almost equal to a 4-ampere arc in brilliancy, and of course much superior in the matter of convenience.

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

THE DEVELOPMENT OF THE HUMAN BODY, A manual of human embryology, J. Playfair McMurrich, A.M., Ph.D., LL.D., Professor of Anatomy in the University of Toronto; formerly Professor of Anatomy in the University of Michigan. Fifth edition, revised and enlarged, with two hundred and eighty-seven illustrations, several of which are printed in colors. Philadelphia, P. Blakiston's Son & Co., 1012 Walnut Street, 1915.

From preface to the fifth edition: The increasing interest in human and mammalian embryology which has characterized the last few years has resulted in many additions to our knowledge of these branches of science, and has necessitated not a few corrections of ideas formerly held. In this fifth edition of this book the attempt has been made to incorporate the results of all important recent contributions upon the topics discussed, and, at the same time, to avoid any considerable increase in the bulk of the volume. Several chapters have, therefore, been largely recast, and the subject matter has been thoroughly revised throughout, so that it is hoped that the book forms an accurate statement of our present knowledge of the development of the human body.

THE USE OF GUIDE PLANES AND PLASTER OF PARIS FOR RECONSTRUCTIONS FROM SERIAL SECTIONS: SOME POINTS ON RECONSTRUCTION

WARREN H. LEWIS

From the Anatomical Laboratory, Johns Hopkins University

FIVE FIGURES

Owing to the generosity of the Carnegie Institution of Washington, funds were made available for reconstructing the head of a 21 mm. human embryo, No. 460, Mall collection. During the process of this reconstruction, some rather helpful methods were developed, which others who are engaged in similar work may find useful, and with this in view I have been urged to publish the methods employed.

In regard to the preservation and staining of the embryos, as well as the cutting and mounting, it is assumed that these operations have been carried out in such a manner that the series is practically perfect, and that no unavoidable shrinkage has occurred during the dehydration and embedding; and that in cutting the orientation is such as to give as nearly perfect horizontal, sagittal or frontal sections as is possible. Great care must be taken in mounting the sections on the slides in order to avoid distortions. Reconstruction is undoubtedly greatly facilitated by the use of guide lines in the sections, such as are produced by the ordinary camphor black method. Unfortunately, such guide marks were not present on any of the series of sections used and it was necessary to depend for the form on photographs or camera drawing of the whole embryo, made before cutting. These should be as nearly as possible from lateral, frontal at horizontal views.

PHOTOGRAPHS OF SECTIONS

I have been able to abandon the laborious and time-consuming method of drawing or tracing each section projected in the usual manner onto paper, and have substituted the more expensive but far better method of photographing on large plates, and using the line bromide or azo G hard (matte) prints. While this method is more expensive as regards the immediate outlay of money, it is much cheaper in the end than the old method of tracing, when account is made of the time involved. The photographs are far superior to any drawing that can possibly be made and greatly facilitate the work, both on account of

the greater accuracy and the greater wealth of detail. The various structures to be reconstructed may first be colored on the photograph, thus enhancing the clearness and sharpness of the picture. The ordinary Sussner creta polycolor pencils were used. The photographs of the sections were made in a dark-room with the ordinary Zeiss projection apparatus and for ordinary diameters, 40 or 50, the Zeiss 5 cm. planar lens was used. This is an ideal lens for such work since there is no measurable distortion of the image. In the place of an ordinary arc lamp, we substituted a 250-watt mazda stereopticon bulb, a round bulb with filaments grouped together in a small ball at the center. To avoid unequal illumination from the spiral filaments a ground glass plate was interposed directly in front of the light. The advantages of the mazda light over the arc are (1) it remains constant, and (2) it can easily be turned on or off for time exposures.

The arrangement of the Zeiss optical bench is shown by the diagram, figure 1. I am aware that this may not be the most perfect arrangement, still we were able to obtain excellent results. The most important point consists in focusing each section by moving the slide carrier back and forth, the lens remaining fixed, instead of the usual method of moving the lens back and forth. When the magnification is once adjusted to the required diameter, the lens (7) is securely fixed in position and the plate-holder (14) likewise. Thereafter, the object or slide is brought into focus by means of a fine adjustment connected with the focusing-rod (10). Magnification is thus not altered from section to section or from slide to slide, since the sections are thus brought in the focus of the lens. This insures equal magnification of every section, the most important condition for accurate reconstruction. This method of focusing was introduced into the laboratory by Doctor Essick.

The dark-room by this method becomes the camera, in which a perpendicular board (14) with slots for the plates takes the place of the plate-holder. This board is pivoted in the center and can be freely turned at any angle in a perpendicular plane and clamped there by means of a thumb-screw at the back.

The plate-holder-board is attached to a movable stand, which can be moved to or from the lens in a straight line only, and can be securely clamped in position when in the proper place. At 50 diameters' magnification with the 50 mm. planar lens the plate-holder should be about 6 ft. and 8 in. from the lens. A series of holes can be so placed on the plate-board, as at a, b, c, figure 1, into which the clamp bolt can be changed and the board given a new center for different sized plates. An old plate having the same thickness as those about to be exposed was used for focusing, after having had a fine piece of white paper tightly pasted over it. An undeveloped plate is still better.

Standard orthonon and Stanley commercial plates were used. The latter are apparently just as good and much cheaper than the former. An exposure of about 5 seconds gave the best results; diaphragm of the planar lens at 4. Flat negatives with very little contrast give the best

prints, while 'contrasty' plates, which appear very beautiful, give very poor prints since the shadows and high lights are in too strong contrast. When the shadows and high lights are in strong contrast in the sections—as was the case in the series used, since the central nervous system was deeply stained with alum cochineal and the delicate connective tissue but faintly stained—it is not easy to get negatives which will give prints showing detail in both regions. With full development, a strong light and short exposure give flatter, softer negatives than a dim light and long exposure. The diaphragm of the lens should be as wide open as is consistent with a sharp image, to increase light and reduce time of exposure. It is important to use a developer that will help to give softness to the negative and the following formula is recommended. There is a decrease in the amount of sodium carbonate usually employed for the purpose of increasing the softness of the negative:

Formulae for pyro developer

Solution No. 1	Solution No. 2	Solution No. 3.
Water.....470 cc.	Water.....470 cc.	Water.....470 cc.
Oxalic acid....700 mg.	Sod. sulph..... 60 gms.	Sod. carb..... 60 gms.
Pyrogal. acid. 30 gms.		

For tank development use 30 cc. each of solutions 1, 2, 3, to 1000 cc. water; develop 20 minutes at 70° F. For tray use 60 cc. each of solutions 1, 2, 3, to 1000 cc. water; develop 5 minutes at 70° F.

Add 1 drop of a 10% solution of Potassium Bromide to every 30 cc.

Fixing bath formula

Hypo.....	480 gms.
Water.....	1920 cc.

With acid hardener

Water.....150 cc.	Acetic acid (28%).....90 cc.
Sulphite soda.....30 gms.	Powdered alum.....30 gms.

The azo G hard (matte) paper should be given short exposure with bright light and developed with formula recommended for azo portrait prints. If the amount of elon is doubled and the hydrochinon decreased one-half, a still softer print with more detail is obtained. For contrast plates a still softer developer may be used for the prints with the following formula:

Water.....300 cc.	Hydrochinon..... 4 gms.
Elon..... 4 gms.	Carbonate of soda.....22 gms.
Sulphite of soda.....30 gms.	Potassium bromide..... 1 gm.

MODEL OF THE EXTERNAL FORM

With the photographs or drawings of the sections complete, our next step is to make a model of the external form of the embryo or of a large enough part of it to insure that we have as accurate a reproduction as possible. For this the ordinary Born wax plate method is used. Wax plates of the proper thickness are cut out for the external form and piled either by the orientier guide lines or according to the photographs of the external form. It is extremely important that this external form shall be as perfect as possible, for on it all subsequent reconstructions are based, as will be seen later.

The wax plates should be piled without fusing so that they can be easily unpiled later. In making this external form of a whole embryo, it is usually best to begin piling with the larger plates from the middle of the trunk towards the top of the head, and another pile from the same region towards the tail, as when horizontal sections are used. In fact, two or three piles may be used, provided the guiding curves coincide. Or one may pile the head with the trunk, then lift off the head and turn the trunk piece upside down and pile the tail end on it. In this case the guide curves will overlap.

It is a great help to use a guide curve from a negative outline in cardboard of the external form of the embryo, made by magnifying the photograph of the external form to the proper diameter, as shown in figure 2. In horizontal series this curve should be made from a direct sagittal view of the embryo, and will of course be in the same plane as the median sagittal plane of the model. It is also important to establish the relation of the plane of the sections to this enlarged outline in order to give the proper angle to the guide curve.

The sections of different embryos, for example, may be cut at somewhat different angles to the *frontal plane* of the embryo, as in figure 3. So if one were piling the head end of the embryo from the middle of the trunk, it would be necessary to determine the plane of the sections in relation to the whole embryo, whether in the direction of *a* or *b* or *c*, etc., in order that the base-line of the cardboard guide curve may coincide with the proper plane *a* or *b* or *c*, etc., when it rests on the base-board. It may also happen that the sections in a horizontal series are cut obliquely to the median sagittal plane, as is often the case. In piling the plates for the external form from such a series the median sagittal plane of the plates must be made to start at a corresponding angle to the base-board, leaning either in the right or left, as the case may be. In this way the plates are piled up with their flat surfaces parallel to a horizontal base-board.

In a similar manner, the proper angle should be used in piling plates from sections cut more or less obliquely to the other planes of the embryo. It is important that the piling be done on a board with a true surface. Overhanging parts which are likely to sag should be supported from the base-board.

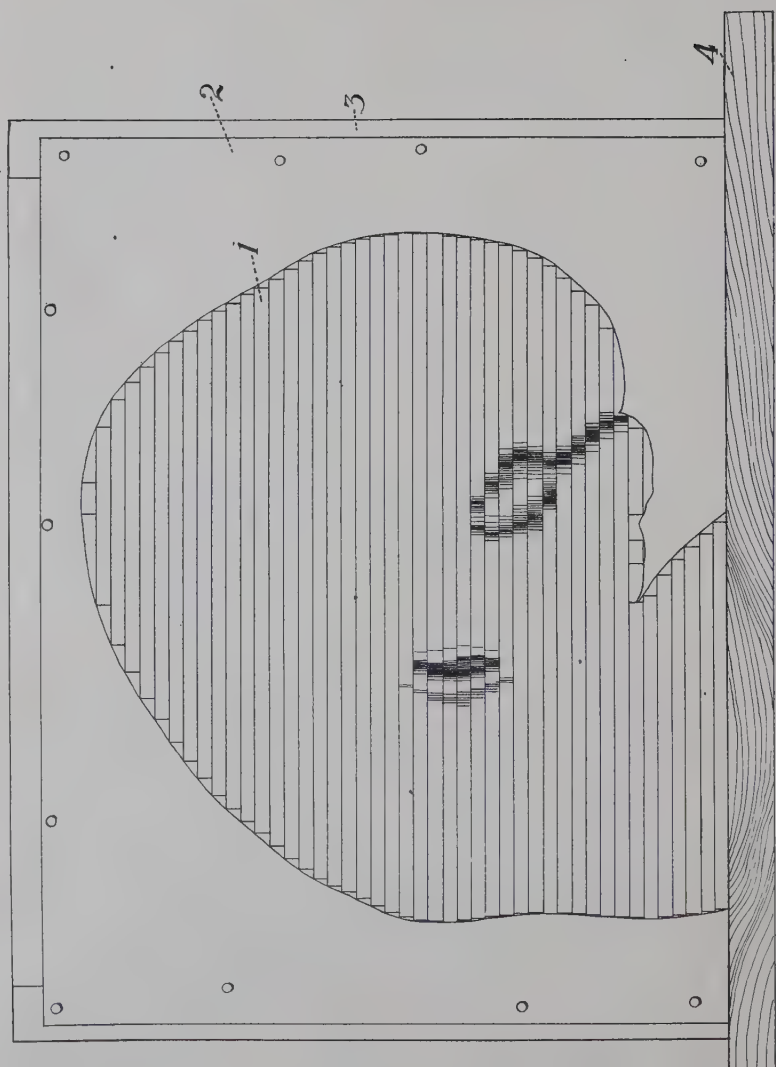


Fig. 2 Method of piling with cardboard outline as guide; 1, external form piled in wax plates; 2, cardboard outline guide; 3, posts for attaching same; 4, baseboard.

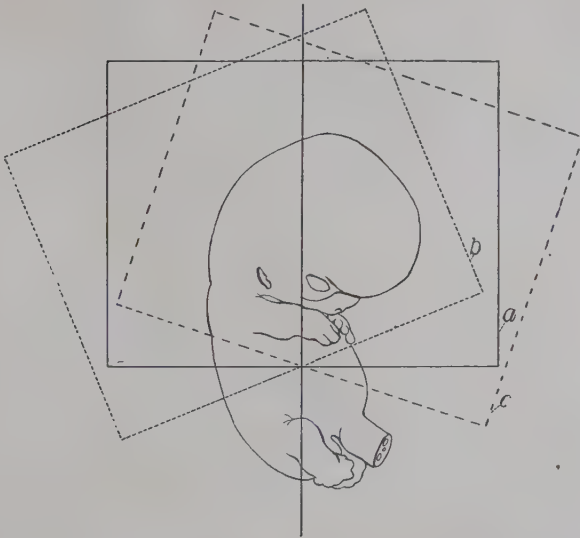


Fig. 3 Outline of embryo to show different arrangements of cardboard guide *a*, *b*, *c*, for cross-sections, cut at different angles to the frontal plane of the embryo.

ESTABLISHING GUIDE PLANES

Since in most series there are no guide marks, it was necessary in some manner to establish guide lines on our photographs that could be used for all subsequent reconstructions. I first thought of drilling two perpendicular holes through the entire series of plates as they stood together in the piled-up form, and then by placing each plate on its own photograph the position of the hole could be traced onto the photograph and we would thus have two orientier marks on each photograph (or drawing) that could be utilized in piling plates for future models.

A somewhat different and better method was finally adopted, which has proved very successful. After the piling of the external form was complete and satisfactory and the cardboard outline removed from the head end (for example, from a series of horizontal sections) two perpendicular posts were erected from the base-board in such a manner that a line drawn between them passed along the median plane of each plate or parallel to it (fig. 4). With a straight-edge rule a line was then drawn with a needle across the top wax plate, the straight edge resting against the edges of the two perpendicular posts, and a second line was drawn at right angles to this at a given distance from one of the perpendicular posts. The top plate was then carefully lifted off and similar lines drawn on the next and each succeeding plate in turn.

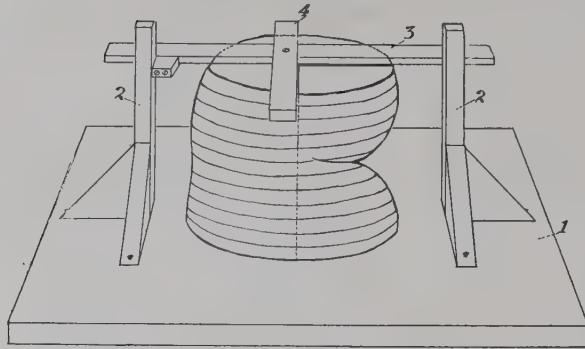


Fig. 4 Method of making guide lines: 1, baseboard; 2, perpendicular posts; 3, straight edge; 4, piece at right angles to it.

Care must be taken, of course, not to disturb the position of the plates until the lines are drawn. Thus there are established on each wax plate two lines, at right angles to each other, which coincide with two planes through the model or embryo that are perpendicular to the plane of the sections. One of the *principal planes* either coincides with the median plane or is parallel to it, while the other is at right angles to this and at a given distance from one of the perpendicular posts. With these two *principal planes* established, it is possible to repile the external form or any other part of the embryo with mathematical precision, since these two planes are likewise perpendicular to the plane of the sections.

After the guide lines have been drawn on each wax plate, they must next be transferred to the photographs or drawings by super-imposing each wax plate on its photograph or drawing and marking at the ends of the guide lines. The wax plate is then lifted off and the points on the photograph connected by lines similar to those on the plates. When the two *principal guide lines* are established we have found it convenient to establish secondary guide lines by drawing other lines, 5 cms. apart, parallel to those over the entire surface of the photographs.

We have introduced lately a still better method: namely the printing of lines in red ink over the photographs from a lithographic stone. These lines form squares of 1 cm. with slightly heavier lines every 5 cms. The lines are printed to correspond with the two principal guide planes. Such lines greatly facilitate not only the plastic reconstruction work but are of especial value for graphic reconstructions. These lines will of course coincide with various planes through the embryo. The advantage in having such a number of planes will become apparent when one wishes to reconstruct small structures that are limited to a particular part of the embryo. The whole section or any part of it will thus be included in rectangles or squares of various sizes depending upon the extent of the part.

WAX MOLD FOR PLASTER OF PARIS CAST

With the development of these guide planes we have abandoned the usual Born wax plate method of making models, and use instead wax plate negatives into which plaster of Paris is poured. The method is as follows: Structures to be modeled are outlined on wax plates in the usual manner, and at the same time, while the plate is still in proper position under the photograph, points are pricked through into the

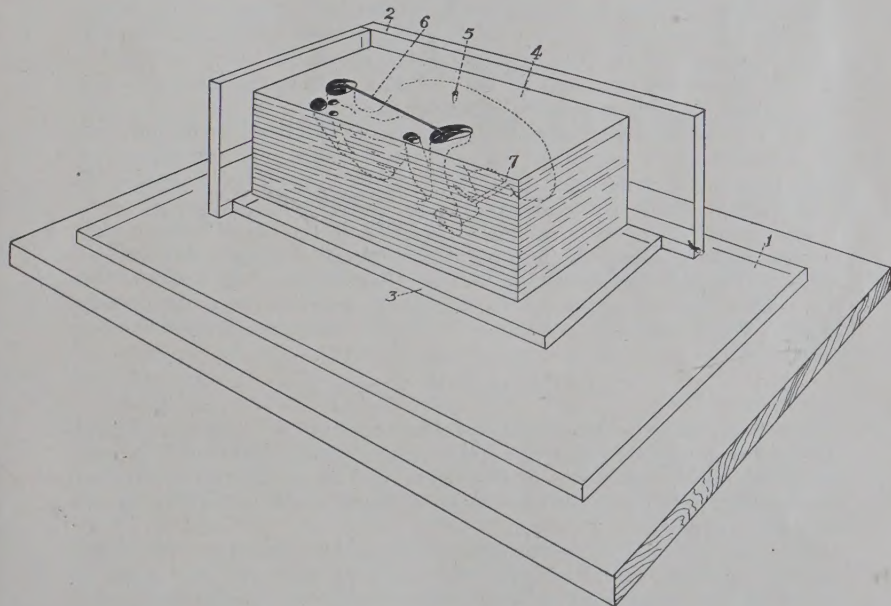


Fig. 5 Method of piling wax mold: 1, baseboard; 2, perpendicular right angle corner; 3, glass plate; 4, wax mold; 5, vent; 6, galvanized iron wire bridge; 7, gate for plaster between parts of mold.

wax with a fine needle at the corners of the rectangle in which the structure or structures outlined are included. The outline is transferred from the photograph onto the wax plate by the use of carbon paper; tracing on the photograph with a smooth glass point. Each plate is then carefully trimmed to the rectangular shape corresponding to that outlined by the four needle points. The outlined structures are then cut out, leaving holes in the plates. The plates are then piled into a perpendicular rectangular corner (fig. 5). Bridges of galvanized iron wire (ordinary iron wire will rust and discolor the cast) can be placed

in position as the piling progresses to hold the various parts of the cast together. Wire, string, or cloth may be inserted into the holes of the finer structures to give strength. Gates and vents to carry plaster from one part of the model to the other and to allow air to escape were cut through suitable places as the piling progressed. It is better not to smooth the inside of the mold. Owing to the fact that the plates were trimmed into rectangles, having the four sides in the same perpendicular planes, the structures which are represented by the holes in the plates must necessarily come into the proper relation with each other. If some of these structures come to the edge of the plate at any place, they would necessarily be cut off by one of these planes. After the piling is completed the outside edges of the plates are fused together to prevent the plaster from leaking out. A wax plate is also fused over the side of the block if any holes come to the edge. In piling the wax plates, it is necessary to measure the height of the block of plates after the addition of each new plate in order to be sure that the plates are piled properly as regards height. It is usually necessary to scrape off a slight burr which comes along the edge of the cut.

We have often found it advisable to build up models in rather small blocks, usually about 50 mm. in thickness, and where the models are large these blocks can be limited in other directions as well and the casts later fused together or merely fitted together as a dissectable model. Such combinations can be varied to suit special conditions.

THE PLASTER OF PARIS CAST

After the piling is completed and the edges of the plates are fused, the bottom of the pile which rests on the glass plate is made fast. Plaster of Paris is then poured into mold until it rises above the top and before it has completely hardened the excess on top is usually scraped off level with the top plate.

We used a grade of plaster known as potter's plaster. The mass consists of about equal weights of water and plaster. The latter is sifted into the water until it just begins to show dry on the top. It is then stirred a little and poured into the mold. After setting for an hour or so, the wax is melted off in boiling water. The cast is taken out and washed in very hot water and dried in the air. Plaster of Paris is wonderful material to work with and requires but little experience to handle it with considerable facility. The plaster cast of course shows the lines of the plates just as wax models do unless considerable polishing is done. It is easier to smooth off a plaster cast than a wax model. The plaster is easily trimmed with a knife or sandpaper and the angles remaining between the edge of the plates can easily be filled in with fresh plaster painted on with a brush to any desired thickness. Corrections and additions can also be made on such plaster models by cutting off and building up with fresh plaster, using wire, string, cloth, etc., if necessary. The different structures are easily tinted with water colors (suspensions in water or ordinary commercial house paint pig-

ments; such as, ultramarine blue, yellow ochre, burnt sienna, chrome yellow, English vermilion, etc.). A better method if one wishes to polish the model, is to mix up fresh plaster by using water colored with the pigment and to do the final smoothing with this mixture. If models are made in sections there is no very great difficulty in putting these together in the proper relation to each other. Finally the entire model can be toughened by soaking in hot paraffin until all the air is driven out by the paraffin which penetrates through the plaster. It is best to have the paraffin bath somewhere between 95 and 100°C. when the model is lifted out, in order that the surface will not be heavily coated with paraffin.

WAX PLATES

The wax plates were made according to the following formula:

Bees' wax.....	6 parts
Paraffin.....	4 parts
White lump rosin.....	2 parts

The ordinary 56°C. Standard Oil paraffin was used; lump rosin is much better than powdered rosin. 2000 grams poured on very hot water—surface 3 by 4 feet—gives plate 2 mm. in thickness. The hotter the wax and water the better. The air bubbles which form in the wax are driven off before the plates cool by playing a Bunsen flame over the surface.

The points which I wish to emphasize are: first, the use of photographs; second, the use of the series of guide lines which coincide with planes that are at right angles to each other and perpendicular to the plane of the sections; and third, the use of plaster of Paris. Although the preliminary steps are somewhat more complicated than those usually employed, they are nevertheless essential and in the end save both time and expense. The advantages of a plaster model are obvious to those who have worked with wax and realize the dangers of distortion and the difficulties involved in strengthening the wax and in modeling fine structures.

MEMOIRS

The publication of this series of Anatomical Monographs has been undertaken with the purpose of presenting the results of original investigation in anatomy which are too extensive for incorporation in the already over-crowded current periodicals.

No. 1. The Anatomy and Development of the Systemic Lymphatic Vessels in the Domestic Cat, by George S. Huntington, Professor of Anatomy, Columbia University, New York City. This monograph states in a few pages the various theories held in regard to lymphatic development in general and then proceeds to present the result of six years' careful investigation on mammalian lymphatic development. Part I deals with the development of the systemic lymphatic vessels in their relation to the blood vascular system. Part II deals with the development of the preazygos and azygos segments of the thoracic duct. This memoir contains 175 pages of text, 8 text figures (two in color), 254 photomicrographs and 21 colored plates. Sent post paid to any country for \$4.00.

No. 2. Contribution to the Study of the Hypophysis Cerebri with Especial Reference to its Comparative Histology, by Frederick Tilney, Associate in Anatomy, Columbia University, New York City. Part I contains a historical review of the literature. Part II deals with the comparative histology of the pituitary gland and gives a report of six hypophysectomies performed upon cats. This memoir contains 72 pages of text, 2 text figures, 60 photomicrographs and plates. Sent post paid to any country for \$1.50.

No. 3. Early Stages of Vasculogenesis in the Cat (*Felis Domestica*) with Especial Reference to the Mesenchymal Origin of Endothelium, by H. Von W. Schulte, Department of Anatomy, Columbia University, New York City. This memoir contains 90 pages of text and 33 figures of which 14 are in colors. Sent post paid to any country for \$1.50.

No. 4. The Development of the Lymphatic System in Fishes, with Especial Reference to its Development in the Trout, by C. F. W. McClure, Department of Comparative Anatomy, Princeton University. In press, to be published in October.

No. 5. The Development of the Albino Rat, *Mus Norvegicus Albinus*: I. From the pronuclear stage to the stage of mesoderm anlage; end of the first to the end of the ninth day: II. Abnormal ova; end of the first to the end of the ninth day; by G. Carl Huber, Department of Anatomy, University of Michigan, and the Division of Embryology, Wistar Institute of Anatomy and Biology, Philadelphia. This memoir contains 142 pages of text and 42 figures from drawings by the author. Sent post paid to any country for \$2.50.

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